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The Characteristics of Korean Traditional Post-Fermented Tea (Chungtaejeon)

Doo-Gyung Moon

Abstract

The structure of the bacterial community involved in the production of oriental traditional post-fermented tea (Chungtaejeon) was investigated using 16S rRNA gene analysis. The main microorganisms in fermentation process of Chungtaejeon are identified as *Pantoea sp.* and *Klebsiella oxytoca*. Phylogenetic analysis suggested that the taxonomic affiliation of the dominant species in the Chungtaejeon was γ -proteobacteria. The bacterial community size was higher about 100 times in Chungtaejeon compared with other Korean tea and puer-tea. Also, the fungal community size was higher about seven times in the Chungtaejeon than in the other post-fermented teas. However, the archaeal community size was highest about six times in the Chungtaejeon. Therefore, the bacteria, fungal and archaeal community sizes were highest for Chungtaejeon than in the seven post-fermented teas. As a result, the microbial communities of Chungtaejeon were the largest compared with other teas. The catechin content decreased from 12.10 to 3.80 mg/g, and epicatechin (EC) and gallic acid contents were increased to 28.50 and 8.02 mg/g, respectively, during manufacturing. The *Pantoea sp.* may perform an important role for manufacturing and fermentation to gallic acid from catechins of Chungtaejeon.

Keywords: EGCG (epigallocatechin gallate), dominant bacteria, gallic acid, γ -proteobacteria

1. The history of Korean tea

Tea plant seeds were brought to Korea from China in 828 CE and planted on Jiri Mount in southern Gyeongsang Province. Korean tea history started from the Three Kingdoms, Goryeo and Joseon Dynasties to present for about 1200 year-old tradition. Now, Korean tea culture has been developed to Korean pottery, tea books, and people who like tea and lantern festivals, etc. However, Korean people employ tea as the symbol for communication, reflection, social justice, loyalty, filial piety and manners, etc. Korean Darye translates to “etiquette for tea,” which is a way of slowing down and relaxing the mind in everyday life with tea. Almost all of South Korea’s tea is grown in the peninsula where people enjoy sea breezes from the Korean strait and the East Sea at the Boseong, Hadong, Jeju and Jangheung regions. Most of the tea produced is green tea picked up from April to May by

hand and machine. Nowadays, green tea (powder green tea), yellow tea, black tea, post-fermented tea and blending tea are produced in the southern region and its consumption have increased every year.

Byeongcha was popular during the Tang Dynasty (China). It is rapidly spread beyond Korean nobles. A small piece of Byeongcha was dug at an ancient tomb of Goguryeo. It is the representative of Korean tea, which has developed uniquely for long time in terms of Korean climate, custom and preferences. The Borimsa temple served as the main during the Three Kingdoms Dynasty. Historical Borimsa records about tea in Jangheung [1]. The Chungtaejeon is post-fermented tea that is a kind of Byeongcha (mold) and looks similar to the coin that is called Doncha (shape).

2. What is Chungtaejeon?

One of the post-fermented teas Chungtaejeon has been developed and inherited from Jangheung in southern coastal areas in Korea over 1200 years ago. It is an international fermented tea, which has a long history from the Three Kingdoms Dynasty to the early modern period of Korea. Chungtaejeon is a kind of Byeongcha (mold). It looks similar to a coin and is called Doncha (shape, **Figure 1**). Also, it is named since its color changes to blue during the fermentation process.

The historical authenticity of Jangheung Chungtaejeon has been recorded on a tombstone in the Borimsa temple. The Borimsa temple served as the main one during the Three Kingdoms Dynasty. On the other hand, Goryeo ran 19 tea spots, and 13 tea spots were in Jangheung [1]. A tea spot is a special production area for tea. Each province ran a national tea farm in the country where tea is well harvested, and the province governor offered tea as a tribute to the king. Also, Jangheung made the best tea of the country during Joseon Dynasty. Nowadays, Chungtaejeon, which is world recognized as a luxury tea, has been approved as registered one for art of taste (2013), selected as “slow food organization” by international life varieties foundation (2014), Japanese international green tea competition best gold medal (2008), gold medal (2011), best gold medal (2014), gold medal (2015 and 2019) and national important agricultural inheritance (2018) [1].



Figure 1.
The shape of Chungtaejeon of Korean traditional post-fermentation.

3. Manufacturing methods of Chungtaejeon

How to make Chungtaejeon? First of all, collect of tea leaves after picking up for one bud two to three leaves in tea garden on the clear day, and then dry for 24 hours in the room of ventilation and removed after selecting to hard leaves and stems. Second, stem the tea leaves in an pot with water vapor for about 5–15 minutes. Third or fifth one, grind it in the large wood mortar until pasting it. Fourth, it in bamboo mold of coin shape and pre-dry. After that, make holes that are 0.2 cm in diameter by needles with bamboo in the center of the mold and dry in the room. Last, ferment it in the pot in the room and store.

Nowadays, people visit Jangheung for their well-being and healing effect by making Chungtaejeon.

4. The characteristics of Chungtaejeon

The post-fermented tea has softer taste, an increased anti-bacterial effect, antioxidant activity, and lower cholesterol production effects by fermentation of microorganisms due to higher amounts of gallic acid, methoxy phenolic compound, and polyflavonoids [2–7]. The Chungtaejeon changes to blue color during the fermentation process (Figure 1).

The raw tea leaves of wild *Camellia sinensis* are dried overnight in a well-ventilated room. The steaming process is carried out for about 15 minutes after removing impurities in the leaves. The steamed tea leaves are pulverized with a motor. After pulverization, the coin-shaped tea balls dry for 2–3 days in a well-ventilated room. The center of coin-shaped tea ball is punched by bamboo stem and connected by a thread to dry it in the sun, ondol room, or shade. Dried Chungtaejeon is kept in a pot to prevent loss of aroma and to avoid moisture. We analyzed bacterial community from raw leaves to products of a 1-year fermented tea (Figure 2).

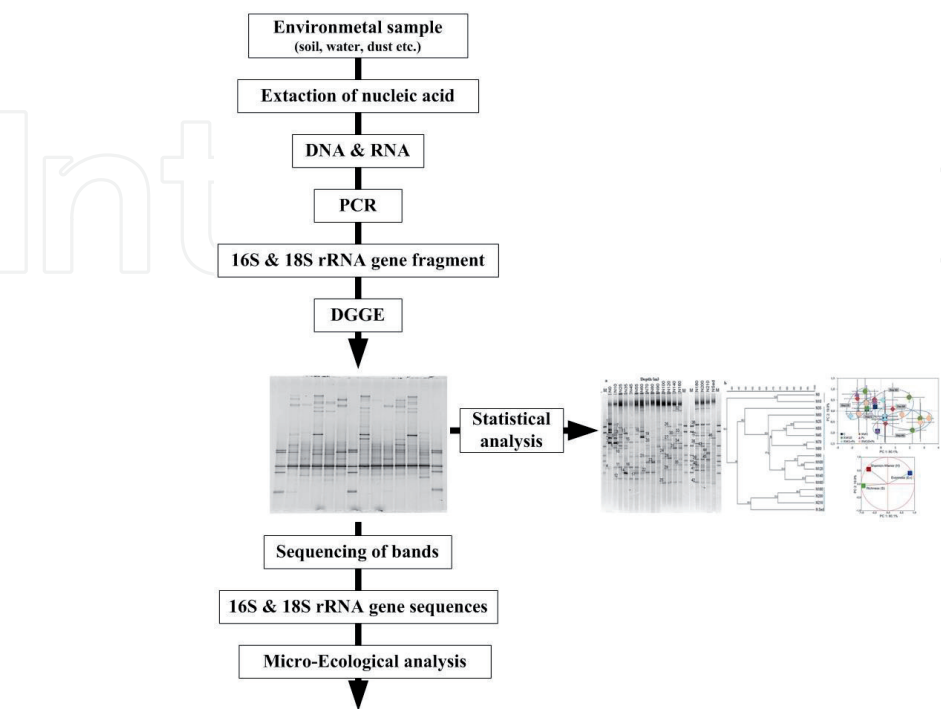


Figure 2.
Flow diagram of PCR-DG-DGGE (double gradient-denaturing gradient gel electrophoresis) analysis for microbial communities.

Item	Before steaming raw leaves	After steaming	After crushing	After forming (0 day)	1 day	3 days	7 days	9 days	11 days	12 days
Acidobacteria	42.86	7.55	2.44	47.6	3.46	7.50	21.4	66.6	13.5	33.3
Alpha-proteobacteria	32.14	1.89	—	—	—	2.50	—	—	—	—
Gamma-proteobacteria	25.0	90.57	97.6	52.4	96.5	90.0	78.5	33.3	86.4	66.6
Total (%)	100	100	100	100	100	100	100	100	100	100

Table 1.
The percentage of the dominant bacteria during manufacturing of Korean traditional post-fermented tea (%).

DNA extractions were carried out in accordance with eukaryotic microalgal nucleic acids extraction (EMNE) method. The bacterial universal primers 27F (5'-GAG TTT GAT CMT GGC TCA G-3') and 518R (5'-WTT ACC GCG GCT GCT GG-3') were used to amplify the 16S rRNA genes for NGS analysis. The primer sequences and PCR conditions for Roche 454 are described in Pitta et al. [8]. After the PCR reaction, purification was carried out using PCR purification kit (Biofact, Daejeon, Korea). NGS was performed with a Roche 454 GS-FLX plus (454 Life Sciences) according to the procedure described by Galan et al. [9]. To compare modified methods with traditional ones for the overall bacterial communities of the Chungtaejeon, operational taxon unit (OTU)-based and phylogeny-based analyses were performed.

Catechins, gallic acid and caffeine contents were analyzed by HPLC (Agilent 1216 Infinity LC) using column (ZORBAX Eclipse plus C18, 4.6 × 250 mm).

We studied the structure of the fermentation bacterial community of Korean traditional post-fermented tea that was investigated by metagenome analysis using 16S rRNA gene during manufacturing (Table 1). The Acidobacteria was changed

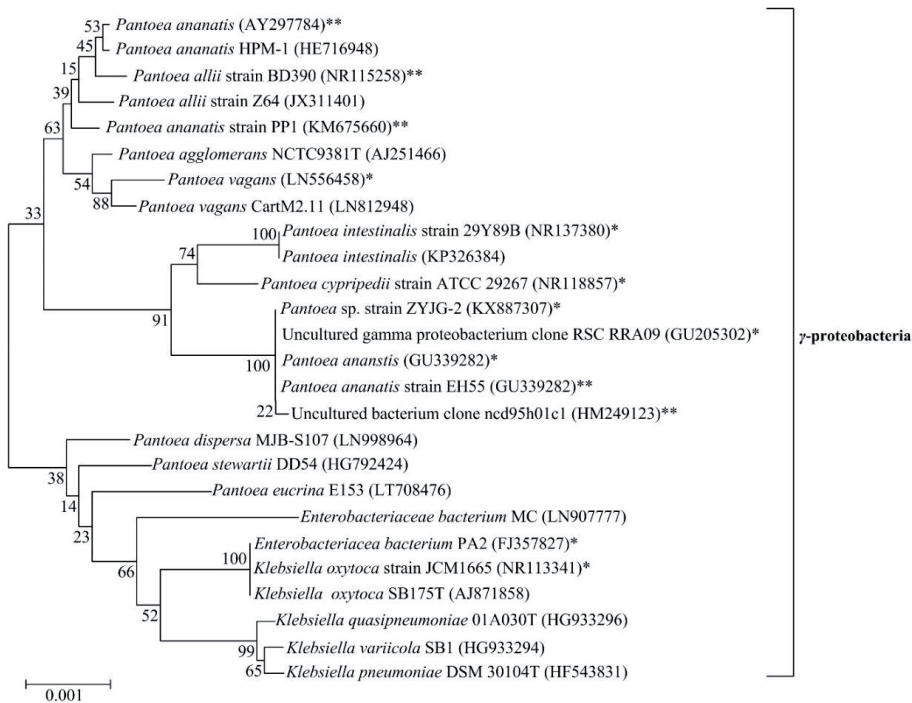


Figure 3. Phylogenetic tree on 16S rRNA gene sequence using the neighbor-joining methods showing the Korean traditional post-fermented tea (Chungtaejeon). *: 16S rRNA gene clone library, **, **: DG-DGGE (double gradient-denaturing gradient gel electrophoresis).

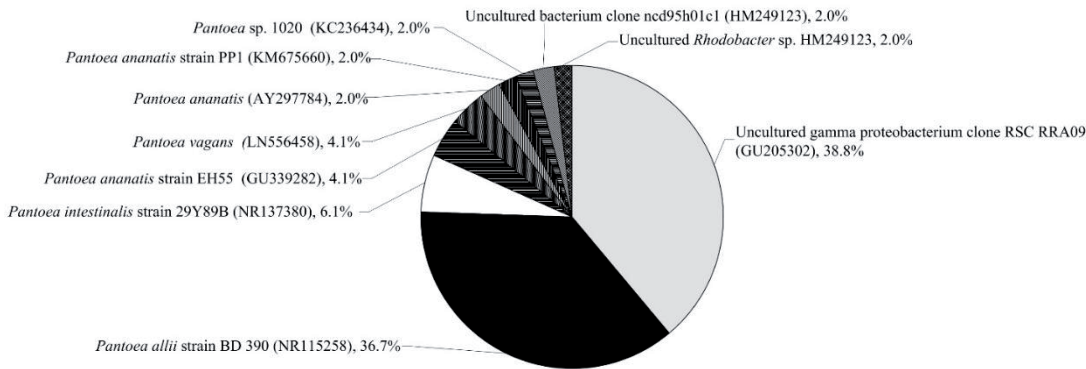


Figure 4. Dominant bacterial OTUs detected in the Korean traditional post-fermented tea (Chungtaejeon) 16S rRNA gene clone libraries.

from 42.86% before steaming raw leaves to 7.55% after steaming and then ranges from 2.44% after crushing to 66.6% (9 day). The alpha-probacteria range from 1.89 to 32.1%. The gamma-probacteria increased from 25.0% before steaming raw leaves to 97.6% after crushing and then from 33.3 to 66.6% during forming and fermentation by manufacturing process. Therefore, dominant bacteria during manufacturing were gamma-probacteria for Chungtaejeon. The microbial community size was the largest for Chungtaejeon compared with other teas [10, 11]. Also, phylogenetic analysis suggested that the taxonomic affiliation of the dominant species in the post-fermented tea was gamma-proteobacteria (**Figure 3**) for fermentation [10]. Our results were similar to the report of Kim et al. [10, 11] during manufacturing of Korean traditional post-fermented tea (**Table 1**).

However, the structure of the bacterial community involved in the production of oriental traditional post-fermented tea (Chungtaejeon) was investigated using 16S rRNA gene analysis (**Figure 2**). The main microorganisms in fermentation process of Chungtaejeon (**Figure 1**) are identified as *Pantoea* sp. and *Klebsiella oxytoca*, respectively (**Figures 3** and **4**). Phylogenetic analysis suggested that the taxonomic affiliation of the dominant species in the Chungtaejeon was γ -proteobacteria (**Figure 3**). Comparison of bacterial (A), fungal (B), and archaeal (C) community

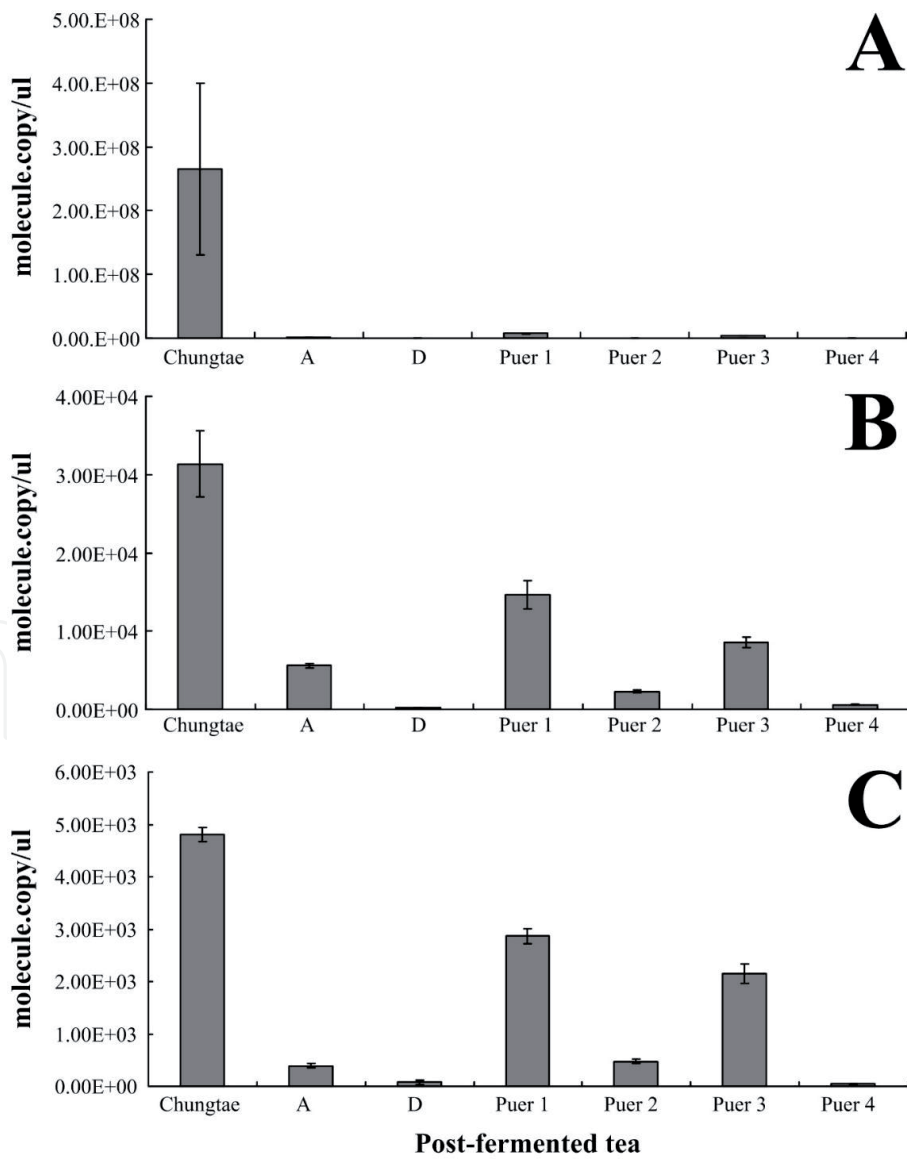


Figure 5. Comparison of bacterial (a), fungal (B), and archaeal (C) community size using the real-time PCR from the various oriental post-fermented tea (Chungtaejeon). A, D and Puer1 ~ 4 were post-fermented tea for Korea and China, respectively.

Taxonomy	Chungtaejeon	Awabancha	Puer 1	Puer 2
Acidobacteria	12.2	0.2	50.0	34.6
Firmicutes	—	44.7	—	26.6
Alpha-proteobacteria	—	5.2	50.0	17.0
Beta-probacteria	1.1	11.5	—	19.3
Epsilonproteobacteria	—	0.1	—	—
Gamma-proteobacteria	86.7	38.3	—	2.5

Table 2.
Dominant microorganism different of oriental post-fermented tea by metagenome analysis (%).

size using the real-time PCR from the various oriental post-fermented tea (Chungtaejeon), other Korean post-fermented tea and puer-tea from China is shown in **Figure 5**. The bacterial community size was the highest for Chungtaejeon. It is higher about 100 times in Chungtaejeon ($2.65 \times 10^8 \pm 1.35 \times 10^8$ copy/ul) compared with other Korean tea and puer-tea. Also, the fungal community size was higher about seven times in the Chungtaejeon than in the other post-fermented teas. However, the archaeal community size was highest about six times in the Chungtaejeon. Therefore, the bacteria, fungal and archaeal community size were highest for Chungtaejeon in the seven post-fermented teas. As a result, the microbial communities of Chungtaejeon were the largest compared with other teas.

Also, cluster analysis confirmed that microbial population present in both Korean and Chinese post-fermented teas groups into the same class [11]. The dominant microorganism present in Korean post-fermented tea was bacterium, while for the Chinese post-fermented tea, it was fungus [5–7, 11]. However, dominant microorganism different from oriental post-fermented tea by metagenome analysis was a acidobacteria and alpha-proteobacteria for Puer 1 and Puer 2, firmicutes for Awabancha and gamma-proteobacteria for Chungtaejeon (**Table 2**).

Tea is classified as green tea, semi-oxidation tea, oxidation tea and post-fermented tea, depending on its manufacturing methods. Post-fermented tea has softer taste, an increased anti-bacterial effect, antioxidant activity, and lower cholesterol production effects by fermentation of microorganisms due to higher amounts of gallic acid. The gallic acid content was 1.67 mg/g for green tea and 21.98 mg/g for puer-tea [4, 12–15].

5. The beneficial effects of Chungtaejeon

Tea is one of the most popular beverage that is produced from the tea plant, *Camellia sinensis* (L.) O. Kuntze and is consumed as green, black, Oolong and post-fermented tea in different parts of the world. The beneficial effects of green tea such as cancer, heart disease, and liver disease are related to catechins [16]. Black tea has made promising pharmacological effects such as growth promoter, cardioprotector, potent cholesterol-lowering effect, antioxidant and antimicrobial, etc. in humans for various compounds such as flavonoids (Thearubigins and theaflavins and catechins), amino acid (L. theanine), phenolic acids (gallic acid and caffeic acid etc.), vitamins, etc. [17]. The Chungtaejeon is post-fermented tea that is produced in Korea by microorganisms. Chemical analysis of it demonstrated the presence of tannins, flavonoids, glycosides, vitamins, polysaccharides, and volatile oils [18]. Also, Chungtaejeon possessed strong antioxidative effects and effectively inhibited the cytokine that induces proliferation. Furthermore, it prevents migration of human aortic smooth muscle cells (HASMC) by restraining the protein expression and enzymatic action of matrix metalloproteinases (MMP-9) [19].

The dominant bacteria, catechins and gallic acid contents during Chungtaejeon marking process are analyzed from raw leaves to product. The catechin content decreased from 12.10 to 3.80 mg/g, and epicatechin (EC) and gallic acid contents were increased to 28.50 and 8.02 mg/g, respectively, during manufacturing (**Figure 3**). Microorganism oxidizes phenolic compounds of tea and leads to considerable loss of the catechins and formation of the theaflavins, thearubigins, theabrownins and gallic acid [4–7, 13–15]. Our results are similar to decreased catechin content and increased gallic acid content during manufacturing and fermentation. Lee et al. [20] also report that levels of EC, ECG, EGCG, quinate, caffeine and sucrose decreased, whereas gallate and glucose levels increased during tea fermentation. Epigallocatechin-3-gallate (EGCG) content was the highest of 90.2 ± 16.1 mg/g for catechins (**Figure 6**). EGCG was the major catechin among all tea varieties, accounting for about half of the total catechins. Epigallocatechin-3-gallate is the ester of epigallocatechin and gallic acid. EGCG in tea, which is made to green tea, white tea and fermented tea, is a polyphenol under basic research for its potential to affect human health and disease such as dietary supplements. The catechins such as EGCG most changed to gallic acid during fermentation into puer tea [4–7, 12, 14, 15]. The most important pharmacological properties of gallic acid are attributed to its antioxidant and anti-inflammatory potentials [12].

Pantoea agglomerans has been concerned to decompose polyphenol tannic acid and gallic acid [4–6, 15]. It may perform an important role for manufacturing and fermentation into gallic acid from catechins of Chungtaejeon (**Figures 3 and 6**). The gallic acid is the most common member of phenolic acids. Gallic acid or 3,4,5-trihydroxybenzoic acid is one of the most abundant phenolic acids in the plant kingdom such as black tea and post-fermented tea products. The edible use of gallic acid and its ester derivatives is on diverse scientific reports on biological and pharmacological activities of these phytochemicals for antioxidant, antimicrobial, anti-inflammatory, anticancer, cardioprotective, gastroprotective and neuroprotective effects [21]. Also, gallic acid and its derivatives demonstrated a broad range of beneficial effects in prevention and/or management of several disorders, also their acceptable

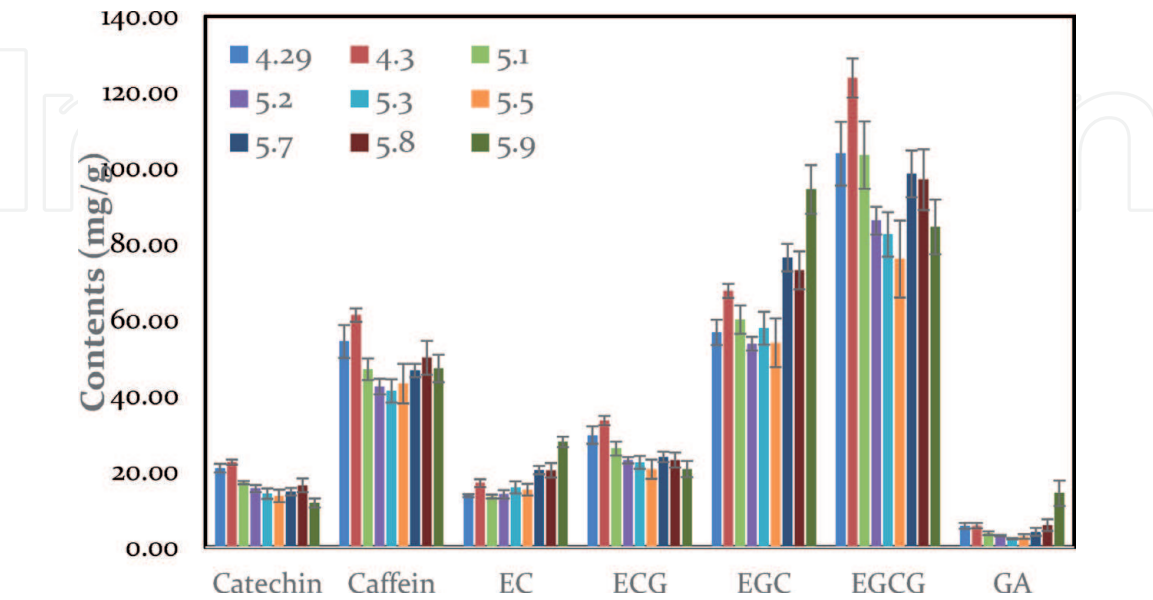


Figure 6. Catechins, gallic acid and caffeine contents after forming of Korean traditional post-fermented tea (mg/g). Forming time (4.29), 1 day (4.30), 2 days (5.1), 3 days (5.2). 5 days (5.3), 7 days (5.5), 8 days (5.7), 9 days (5.8) and 10 days (5.9). EC (epicatechin), ECG (epicatechin gallate), EGC (epigallocatechin), EGCG (epigallocatechin gallate).

safety and stability profiles, making them significant options to be introduced as dietary supplements [22] like *Camellia sinensis*. Black tea also contains gallic acid.

Black tea has many kinds of biological compounds such as flavonoids [thea-rubigins (TRs) and theaflavins (TFs) and catechins, amino acid (L. theanine), vitamins (A, C, K), phenolic acids (caffeic acid (CA), gallic acid (GA), chlorogenic acid (CGA) and carbamic acid], lipids, proteins, volatile compounds, carbohydrates, β -carotene and fluoride that are illustrated as having many promising pharmacological effects as growth promoter and cardioprotector, having potent cholesterol-lowering effect, and being antioxidant and antimicrobial for humans [17]. However, Chungtaejeon can be suggested to have beneficial effect in the prevention of atherosclerosis [8, 10, 11, 17–20, 22, 23]. It prevents the risk of atherosclerosis in rats fed a high-fat atherogenic diet *in vivo and vitro* [23]. The chemical composition of tea leaves consists of tanning substances, flavonols, alkaloids, amino acids, enzymes, aroma-forming substances, vitamins, minerals and trace elements contained in theaflavins (TFs), thearubigins (TRs) and theabrownins (TBs) needed to analyze during manufacturing and fermentation of Chungtaejeon and needed to reveal molecular level such as pharmacological value and therapeutic properties for human health in the future.

6. How to drink Chungtaejeon

Tea utensils such as brazier, pipkin, bamboo chopsticks, teacup and cooling bowl are necessary before boiling and brewing. Also, you can add one piece of Chungtaejeon in one pot (about 1 L) before boiling [1]. There are generally two methods for drinking. The first way to drink Chungtaejeon is drinking after boiling. You can turn leaves into roasted ones in gentle heat for 3–4 minutes. It has sterilization effect, and it adds savory flavor and is a unique flavor. And then, you can add Chungtaejeon leaves split in a liter of boiled water. After you boil it for more than 5–4 minutes, you can have it thoroughly infused. Therefore, you pour boiled water in the tea kettle, and you can add Chungtaejeon leaves split and infuse tea sufficiency. Also, you can have it with ginger, yuzu, quince and herbal medicine depending on your preference. The second way to drink Chungtaejeon is drinking after brewing as follows; roast heat pottery, brassware, and soup bowl or roof file slowly and turn them into golden brown so that can be boiled to drink, on the other hand, the green smell of tea will diminish and unique aroma and flavor will deepen if it is lightly roasted. Also, the color of tea gets better and foreign substances can be eliminated. But, tea should be roasted, not burned. Divide it into small pieces (division into 3–4 parts) so that roasted Chungtaejeon can be easily brewed. And then, pour about 500–600 ml of boiling water at 100°C, and then leave it for more than 10 minutes. After that, drink brewed tea using a cup. It can be brewed about 3–5 times more.

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